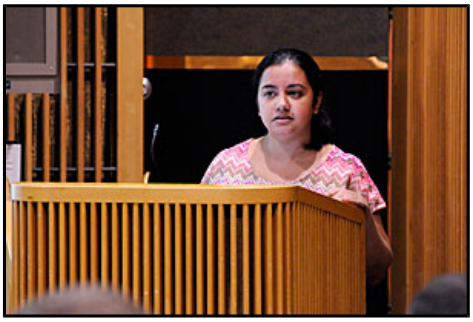
DNA glycosylases in action - the search for damaged bases

By Raj Gosavi

Microbiologist and cancer researcher Susan Wallace, Ph.D., *(http://www.uvm.edu/~vase/?Page=members/directory/WallaceS.html)* described her latest findings on how DNA glycosylases locate and repair damaged substrates, a process that may ultimately affect cancer susceptibility and carcinogenesis, in a talk Aug. 5 at NIEHS.



NIEHS postdoctoral fellow Deepa Singh, Ph.D., above, hosted the talk on behalf of the Laboratory of Molecular Genetics Trainees' Action Committee. In her introduction, Singh remarked that Wallace was a good mentor, and took great interest in the career and professional development of graduate students and postdoctoral fellows in her lab. (Photo courtesy of Steve McCaw)

Aptly titled "DNA glycosylases search for and destroy oxidized DNA bases," Wallace's presentation explored the routine, endogenous production of oxidized DNA damage or lesions by exposure to radiation, inflammation, or chemical agents that happens six to ten thousand times each day. Most endogenous damages are effectively removed by a repair process called base excision repair.

Wallace is a distinguished professor and chair of the Department of Microbiology and Molecular Genetics at the University of Vermont (UV). She has received many honors for her work in the fields of cancer and molecular biology, has authored more than 170 publications and book chapters, and is currently an associate editor of the journals DNA Repair and Molecular Cancer Research.

Wallace set the tone for her talk, with a comment designed to underscore the importance of her field of study. "Fortunately, we have a well-functioning base excision repair pathway," she said. "Otherwise we all wouldn't be sitting here today."

Wallace, who has been consistently funded by the National Cancer Institute, has made significant contributions to biomedical science and, specifically, the field of radiation research (see UV feature story) (http://www.uvm.edu/microbiology/suan-wallace-feature-story.pdf) for over four decades. (Photo courtesy of Steve McCaw)

Lesions worth repairing

Wallace's research focus *(http://www.uvm.edu/microbiology/wallace-lab/)* is to understand basic principles of how DNA glycosylases scan, recognize, and process their substrates, using a combination of techniques, such as enzyme kinetics and X-ray crystallography. By better understanding this critical process in the survival of the organism, she is searching for insights into how and why it goes wrong, and, conceivably, some way to help get repair back on the right track.

The base excision repair pathway is central in repair of these oxidized DNA lesions. The first step in this crucial pathway is where DNA glycosylases come into play. As Wallace explained, DNA glycosylases search for lesions and, once found, they kink the DNA, flip the lesion into the active site, and cleave the glycosyl bond.

Bacteria to human

One interesting aspect of Wallace's studies involved DNA glycosylases from plants, fungi, and vertebrates, because the key proteins do not function quite the same way in bacteria, yeast, or nematodes. Studying the similarities and differences among these glycosylases was important in providing some insight into their substrate specificities. Bacteria primarily use the Fpg family of proteins to remove 8-oxoguanine lesions, with the help of a region on protein called lesion recognition loop. Analogous glycosylases from plants (Fpg-like) and mammalians (NEIL1) have this region missing and consistently did not recognize the 8-oxoguanine lesions on DNA.

Wallace noted that NEIL2 and NEIL3, two other Fpg-like proteins in mammals, had even more diverse characteristics. Both preferred lesions in single-stranded DNA, because they lacked a couple of residues that are important in flipping the lesion bases out of the duplex DNA.

In the process of understanding the nature of broad substrate specificity of glycosylases, Wallace used crystal structures of bacterial glycosylases (Fpg/Nei family of proteins) with the lesion in the active site, and found something she wasn't expecting. "The binding pocket had very few interactions with the lesion," she explained. "Our results suggest that glycosylases need to detect the lesion before binding."

Walking a tight rope

Wallace then looked towards single molecular studies to understand how these enzymes detected lesions on DNA. Her work showed short videos of glycosylase molecules moving back and forth on a piece of DNA stretched between two polylysine beads. This experiment allowed the observation of different diffusion patterns, or movements, of glycosylases on DNA, such as pause, slow, fast, and very fast. Wallace then wondered if the slow and fast diffusion behavior meant glycosylase was trying to find the lesion.

Upon further investigation, she found a residue on glyosylases, called a wedge residue that was involved in scanning the DNA to find the 8-oxoguanine lesions. These studies confirmed the hypothesis that glycosylases have a mechanism to scan the DNA looking for lesions.

(Raj Gosavi, Ph.D., is a research fellow in the NIEHS Structure and Function Research Group.)

The audience included several senior scientists, such as NIEHS lead researcher William Copeland, Ph.D., left, head of the Mitochondrial DNA Replication Group, and retired NTP geneticist Jack Bishop, Ph.D. (Photo courtesy of Steve McCaw)

Postdoctoral fellow Steven Roberts, Ph.D., was among the many trainees in the audience who turned out to hear a giant in the field of DNA repair. (Photo courtesy of Steve McCaw)

The Environmental Factor is produced monthly by the National Institute of Environmental Health Sciences (NIEHS) *(http://www.niehs.nih.gov/)*
, Office of Communications and Public Liaison. We welcome your comments and suggestions. *(bruskec@niehs.nih.gov)*